

Application Serial No. 10/630,968  
Amendment dated 2 June 2011  
Reply to Office Action dated 15 March 2011

*AMENDMENTS TO THE CLAIMS*

This listing of claims will replace all prior versions, and listings, of claims in the application.

*Listing of Claims*

Claims 1-2 (canceled).

Claim 3 (previously presented): The method of claim 33, wherein the mammalian promoter is a Pol III promoter.

Claim 4 (original): The method of claim 3, wherein the Pol III promoter is a mammalian U6 promoter.

Claim 5 (original): The method of claim 4, wherein the U6 promoter is a human U6 promoter.

Claim 6 (previously presented): The method of claim 33, wherein the sequence encoding the terminator sequence comprises a sequence of about 4-6 deoxyadenosines.

Claim 7 (original): The method of claim 6, wherein the sequence encoding the terminator sequence comprises a sequence of 6 deoxyadenosines.

Claim 8 (previously presented): The method of claim 33, wherein the second oligonucleotide primer further comprises a tag sequence to identify functional siRNA encoding sequences.

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Claim 9 (original): The method of claim 8, wherein the tag sequence further comprises a restriction site useful for cloning.

Claims 10-16 (canceled).

Claim 17 (currently amended): The method of claim 36, further comprising the step of transfecting a mammalian cell *in vitro* with the first amplified mammalian promoter-containing siRNA expression cassette and with the second amplified mammalian promoter-containing siRNA expression cassette, wherein the sense strand of the double stranded siRNA molecule and the antisense strand of the double stranded siRNA molecule are expressed in the transfected cell, whereby a double stranded siRNA molecule is produced in the transfected cell.

Claim 18 (canceled).

Claim 19 (original): The method of claim 17, wherein one or more of the oligonucleotide primers are modified.

Claim 20 (original): The method of claim 19, wherein one or more of the oligonucleotide primers are modified by phosphorylation.

Claim 21 (previously presented): The method of claim 17, further comprising the step of screening for a target site on mRNA sensitive to the double stranded siRNA molecule produced.

Claims 22-32 (canceled).

Claim 33 (currently amended): An amplification-based method for producing [[a]] at least one mammalian promoter-containing siRNA-expression cassette, comprising:

- (i) adding a double stranded nucleic acid comprising a mammalian promoter to an amplification reaction mixture, wherein the double stranded nucleic acid has a sense strand and an antisense strand and wherein each of the sense strand and antisense strand has a 5' end and a 3' end, wherein the mammalian promoter is capable of transcribing an RNA molecule in mammalian cells;
- (ii) adding a first oligonucleotide primer to the reaction mixture, wherein the first oligonucleotide primer is complementary to the 3' end of the antisense strand of the double stranded nucleic acid;
- (iii) adding a second oligonucleotide primer to the reaction mixture, wherein the second oligonucleotide primer is complementary to the 3' end of the sense strand of the double stranded nucleic acid and wherein the second oligonucleotide primer comprises a nucleotide sequence that is complementary to a nucleotide sequence that encodes (1) either a sense sequence of a double stranded siRNA molecule ~~or an antisense sequence of the double stranded siRNA molecule~~ and (2) a terminator sequence; and
- (iv) amplifying the double stranded nucleic acid in a polymerase chain reaction amplification comprising (a) annealing the primers to the complementary strands of the double stranded nucleic acid, (b) extending the annealed primers to produce extension products, (c) denaturing the extension products and (d) repeating the polymerase chain reaction amplification steps a sufficient number of times to produce [[an]] a first amplified product comprising [[the]] a first mammalian promoter-containing siRNA expression cassette,  
wherein the first mammalian promoter-containing siRNA expression cassette comprises (1) the mammalian promoter, (2) either the sense strand ~~or the antisense sequence of the double stranded siRNA molecule~~ and (3) the terminator sequence.

Claim 34 (previously presented): The method of claim 33, wherein the amplification product is purified.

Claim 35 (previously presented): The method of claim 33, further comprising cloning the amplified product comprising the mammalian promoter-containing siRNA expression cassette into a cloning vector.

Claim 36 (currently amended): The method of claim 33, ~~wherein two polymerase chain reaction amplifications are performed, wherein a first polymerase chain reaction amplification is performed to produce a first amplified product comprising a first mammalian promoter-containing siRNA expression cassette comprising (1) the mammalian promoter, (2) the sense strand of the double stranded siRNA molecule and (3) the terminator sequence, and wherein further comprising a second polymerase chain reaction amplification is performed comprising:~~

(a) adding a double stranded nucleic acid comprising a mammalian promoter to an amplification reaction mixture, wherein the double stranded nucleic acid has a sense strand and an antisense strand and wherein each of the sense strand and antisense strand has a 5' end and a 3' end, wherein the mammalian promoter is capable of transcribing an RNA molecule in mammalian cells;

(b) adding a first oligonucleotide primer to the reaction mixture, wherein the first oligonucleotide primer is complementary to the 3' end of the antisense strand of the double stranded nucleic acid;

(c) adding a second oligonucleotide primer to the reaction mixture, wherein the second oligonucleotide primer is complementary to the 3' end of the sense strand of the double stranded nucleic acid and wherein the second oligonucleotide primer comprises a nucleotide sequence that is complementary to a nucleotide sequence that encodes (1) an antisense sequence of the double stranded siRNA molecule and (2) a terminator sequence; and

(d) amplifying the double stranded nucleic acid in a polymerase chain reaction amplification comprising (i) annealing the primers to the complementary strands of the double stranded nucleic acid, (ii) extending the annealed primers to produce extension products, (iii) denaturing the extension products and (iv) repeating the polymerase chain reaction

amplification steps a sufficient number of times to produce a second amplified product comprising a second mammalian promoter-containing siRNA expression cassette,

wherein the second mammalian promoter-containing expression cassette comprising comprises (1) the mammalian promoter, (2) the antisense strand of the double stranded siRNA molecule and (3) the terminator sequence.

Claim 37 (previously presented): The method of claim 36, wherein the first and second amplification products are purified.

Claim 38 (currently amended): The method of claim 36, further comprising cloning the first amplified product comprising the first mammalian promoter-containing siRNA expression cassette into a cloning vector and cloning the second amplified product comprising the second mammalian promoter-containing siRNA expression cassette into a cloning vector.

Claim 39 (new): An amplification-based method for producing a mammalian promoter-containing expression cassette, comprising:

(i) adding a double stranded nucleic acid comprising a mammalian promoter to an amplification reaction mixture, wherein the double stranded nucleic acid has a sense strand and an antisense strand and wherein each of the sense strand and antisense strand has a 5' end and a 3' end, wherein the mammalian promoter is capable of transcribing an RNA molecule in mammalian cells;

(ii) adding a first oligonucleotide primer to the reaction mixture, wherein the first oligonucleotide primer is complementary to the 3' end of the antisense strand of the double stranded nucleic acid;

(iii) adding a second oligonucleotide primer to the reaction mixture, wherein the second oligonucleotide primer is complementary to the 3' end of the sense strand of the double stranded nucleic acid and wherein the second oligonucleotide primer comprises a nucleotide sequence that

is complementary to a nucleotide sequence that encodes (1) an antisense sequence of a double stranded siRNA molecule and (2) a terminator sequence; and

(iv) amplifying the double stranded nucleic acid in a polymerase chain reaction amplification comprising (a) annealing the primers to the complementary strands of the double stranded nucleic acid, (b) extending the annealed primers to produce extension products, (c) denaturing the extension products and (d) repeating the polymerase chain reaction amplification steps a sufficient number of times to produce an amplified product comprising a first mammalian promoter-containing expression cassette,

wherein the first mammalian promoter-containing expression cassette comprises (1) the mammalian promoter, (2) the antisense strand of the double stranded siRNA molecule and (3) the terminator sequence.

Claim 40 (new): The method of claim 39, further comprising a second polymerase chain reaction amplification comprising:

(a) adding a double stranded nucleic acid comprising a mammalian promoter to an amplification reaction mixture, wherein the double stranded nucleic acid has a sense strand and an antisense strand and wherein each of the sense strand and antisense strand has a 5' end and a 3' end, wherein the mammalian promoter is capable of transcribing an RNA molecule in mammalian cells;

(b) adding a first oligonucleotide primer to the reaction mixture, wherein the first oligonucleotide primer is complementary to the 3' end of the antisense strand of the double stranded nucleic acid;

(c) adding a second oligonucleotide primer to the reaction mixture, wherein the second oligonucleotide primer is complementary to the 3' end of the sense strand of the double stranded nucleic acid and wherein the second oligonucleotide primer comprises a nucleotide sequence that is complementary to a nucleotide sequence that encodes (1) an sense sequence of the double stranded siRNA molecule and (2) a terminator sequence; and

(d) amplifying the double stranded nucleic acid in a polymerase chain reaction amplification comprising (i) annealing the primers to the complementary strands of the double stranded nucleic acid, (ii) extending the annealed primers to produce extension products, (iii) denaturing the extension products and (iv) repeating the polymerase chain reaction amplification steps a sufficient number of times to produce a second amplified product comprising a second mammalian promoter-containing expression cassette,

wherein the second mammalian promoter-containing expression cassette comprises (1) the mammalian promoter, (2) the sense strand of the double stranded siRNA molecule and (3) the terminator sequence.

Claim 41 (new): The method of claim 40, further comprising the step of transfecting a mammalian cell *in vitro* with the first amplified mammalian promoter-containing expression cassette and with the second amplified mammalian promoter-containing expression cassette, wherein the sense strand of the double stranded siRNA molecule and the antisense strand of the double stranded siRNA molecule are expressed in the transfected cell, whereby a double stranded siRNA molecule is produced in the transfected cell.